Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active RET [658 - 1114]

Enzyme description:- RET [658 – 1114]

Clone number:- DU 58691

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 78, 974.81 daltons
Average Mass 79. 025.60 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.20

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
EAIYAAPFAKKK Final concentration: 300 uM

Division of Signal Transduction Therapy

Clone Data Sheet

RET [658 - 1114]

Protein	RET [658 - 1114]
Clone number	DU 58691
Species	Human
Accession number	P07949-1
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVD**HCY** **HKFAHKPPISSAEMTFRRPAQAFPVSYSSSGARRPSLDSMENQVSVDA** **FKILEDPKWEFPRKNLVLGKTLGEGEFGKVVKATAFHLKGRAGYTTVA** **VKMLKENASPSELRDLLSEFNVLKQVNHPHVIKLYGACSQDGPLLLIV** **EYAKYGSLRGFLRESRKVGPGYLGSGGSRNSSSLDHPDERALTMGDLI** **SFAWQISQGMQYLAEMKLVHRDLAARNILVAEGRKMKISDFGLSRDVY** **EEDSYVKRSQGRIPVKWMAIESLFDHIYTTQSDVWSFGVLLWEIVTLG** **GNPYPGIPPERLFNLLKTGHRMERPDNCSEEMYRLMLQCWKQEPDKRP** **VFADISKDLEKMMVKRRDYLDLAASTPSDSLIYDDGLSEEETPLVDCN** **NAPLPRALPSTWIENKLYGMSDPNWPGESPVPLTRADGTNTGFPRYPN** **DSVYANWMLSPSAAKLMDTFDS**
Native sequence	Amino acids H658 – S1114 (end) of human RET. Residue H238 of the fusion protein is equivalent to H658 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228
Cloning sites	*Sal*1 and *Not*1 sites of pFastBac GST 6P3

Nucleotide sequence of insert

gtcgacCACTGCTACCACAAGTTTGCCCACAAGCCACCCATCTCCTCA
GCTGAGATGACCTTCCGGAGGCCCGCCCAGGCCTTCCCGGTCAGCTAC
TCCTCTTCCGGTGCCCGCCGGCCCTCGCTGGACTCCATGGAGAACCAG
GTCTCCGTGGATGCCTTCAAGATCCTGGAGGATCCAAAGTGGGAATTC
CCTCGGAAGAACTTGGTTCTTGGAAAAACTCTAGGAGAAGGCGAATTT
GGAAAAGTGGTCAAGGCAACGGCCTTCCATCTGAAAGGCAGAGCAGGG
TACACCACGGTGGCCGTGAAGATGCTGAAAGAGAACGCCTCCCCGAGT
GAGCTGCGAGACCTGCTGTCAGAGTTCAACGTCCTGAAGCAGGTCAAC
CACCCACATGTCATCAAATTGTATGGGGCCTGCAGCCAGGATGGCCCG
CTCCTCCTCATCGTGGAGTACGCCAAATACGGCTCCCTGCGGGGCTTC
CTCCGCGAGAGCCGCAAAGTGGGGCCTGGCTACCTGGGCAGTGGAGGC
AGCCGCAACTCCAGCTCCCTGGACCACCCGGATGAGCGGGCCCTCACC
ATGGGCGACCTCATCTCATTTGCCTGGCAGATCTCACAGGGGATGCAG
TATCTGGCCGAGATGAAGCTCGTTCATCGGGACTTGGCAGCCAGAAAC
ATCCTGGTAGCTGAGGGGCGGAAGATGAAGATTTCGGATTTCGGCTTG
TCCCGAGATGTTTATGAAGAGGATTCCTACGTGAAGAGGAGCCAGGGT
CGGATTCCAGTTAAATGGATGGCAATTGAATCCCTTTTTGATCATATC
TACACCACGCAAAGTGATGTATGGTCTTTTGGTGTCCTGCTGTGGGAG
ATCGTGACCCTAGGGGGAAACCCCTATCCTGGGATTCCTCCTGAGCGG
CTCTTCAACCTTCTGAAGACCGGCCACCGGATGGAGAGGCCAGACAAC
TGCAGCGAGGAGATGTACCGCCTGATGCTGCAATGCTGGAAGCAGGAG
CCGGACAAAAGGCCGGTGTTTGCGGACATCAGCAAAGACCTGGAGAAG
ATGATGGTTAAGAGGAGAGACTACTTGGACCTTGCGGCGTCCACTCCA
TCTGACTCCCTGATTTATGACGACGGCCTCTCAGAGGAGGAGACACCG
CTGGTGGACTGTAATAATGCCCCCCTCCCTCGAGCCCTCCCTTCCACA
TGGATTGAAAACAAACTCTATGGCATGTCAGACCCGAACTGGCCTGGA
GAGAGTCCTGTACCACTCACGAGAGCTGATGGCACTAACACTGGGTTT
CCAAGATATCCAAATGATAGTGTATATGCTAACTGGATGCTTTCACCC
TCAGCGGCAAAATTAATGGACACGTTTGATAGTtaagcggccgc